

WHAT IS CLAIMED

1. A method for *in situ* formation of a superficial cartilage layer over an articular cartilage lesion, said method  
5 comprising steps:

a) obtaining a piece of autologous or heterologous cartilage;

b) isolating, culturing and expanding chondrocytes into a neo-cartilage;

10 c) preparing a neo-cartilage construct;

d) implanting said neo-cartilage construct into said lesion; and

e) depositing a top biocompatible adhesive sealant over the neo-cartilage construct.

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2. The method of claim 1 additionally comprising a step of depositing a biocompatible adhesive sealant at a bottom of the lesion wherein said bottom and top sealant may be the same or different.

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3. The method of claim 2 wherein said neo-cartilage construct comprises neo-cartilage chondrocytes embedded into a support matrix.

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4. The method of claim 3 wherein said support matrix is a two or three-dimensional structure prepared from a compound selected from the group consisting of a thermo-reversible gelation hydrogel, collagenous gel, Type I collagen, Type II collagen, Type IV collagen, gelatin, agarose, a cell-contracted  
30 collagen containing proteoglycan, a cell-contracted collagen containing glycosaminoglycan, a cell-contracted collagen containing glycoprotein, fibronectin, laminin, a growth factor, cytokine, elastin, hyaluronin, fibrin, a synthetic polymeric fiber made of polylactic acid, a synthetic polymeric fiber made of  
35 of polyglycolic acid, a synthetic polymeric fiber made of

polyamino acid, polycaprolactone, polyamino acid, polypeptide gel, copolymers thereof and a combination thereof.

5        5. The method of claim 4 wherein said matrix is the  
thermo-reversible gelation hydrogel (TRGH) wherein said TRGH is  
in a liquid sol state at temperatures of below about 30°C and  
wherein said thermoreversible hydrogel polymer is in a solid sol  
state at temperature above about 30°C and further wherein said  
thermo-reversible gelation hydrogel is either deposited into the  
10 lesion cavity formed below the top sealant or between the bottom  
and top sealants as the neo-cartilage construct comprising  
chondrocytes embedded therein or wherein said TRGH is deposited  
into said cavity as a space holding gel without any neo-  
cartilage chondrocytes.

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6. The method of claim 5 wherein the neo-cartilage  
construct comprises cultured differentiated autologous or  
heterologous chondrocytes or cells which could be differentiated  
into chondrocytes, said chondrocytes or cells incorporated into  
20 said support matrix and subjected to an algorithm of the  
invention wherein said algorithm comprises constant or cyclic  
hydrostatic pressure, static atmospheric pressure or non-  
pressure conditions, perfusion flow rate, medium composition,  
temperature, cell density, oxygen concentration and time to  
25 which the chondrocytes are subjected.

7. The method of claim 5 wherein said top and bottom  
sealant is selected from the group consisting of gelatin, a  
copolymer of polyethylene glycol and poly-lactide or poly-  
30 glycolide, periodate-oxidized gelatin, 4-armed pentaerythritol  
thiol and a polyethylene glycol diacrylate, 4-armed tetra-  
succinimidyl ester or tetra-thiol derivatized PEG, photo-  
polymerizable polyethylene glycol-co-poly( $\alpha$ -hydroxy acid)  
diacrylate macromer, 4-armed polyethylene glycol derivatized  
35 with succinimidyl ester and thiol plus methylated collagen

hydrogel, derivatized polyethylene glycol (PEG), derivatized polyethylene glycol (PEG) cross-linked with alkylated collagen, tetra-hydrosuccinimidyl or tetra-thiol derivatized PEG, cross-linked PEG with methylated collagen and a combination thereof  
5 and wherein the top and bottom sealants are the same or different.

8. The method of claim 7 wherein the sealant is cross-linked PEG with methylated collagen.

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9. The method of claim 8 wherein the neo-cartilage construct is prepared *in vitro*, *ex vivo* or *in vivo*.

10. The method of claim 9 wherein the neo-cartilage  
15 construct is prepared *ex vivo* and is subjected to an algorithm of the invention.

11. The method of claim 10 wherein the algorithm  
20 comprises cyclic or constant hydrostatic pressure, static pressure, flow rate, temperature, time, cell density and oxygen and carbon dioxide content.

12. The method of claim 11 wherein the hydrostatic  
25 pressure is from about zero MPa to about 10 MPa above atmospheric pressure at about 0.01 to about 1 Hz, wherein the time for applying the hydrostatic pressure is from zero to about 24 hours per day for from about one day to about ninety days, wherein said hydrostatic pressure is preceded or followed by a period of zero to about 24 hours per day of a static atmospheric  
30 pressure for from about one day to about ninety days, wherein the flow rate is from about 1  $\mu\text{L}/\text{min}$  to about 500  $\mu\text{L}/\text{min}$ , wherein the cell density is from about 3 to 60 millions and wherein the oxygen concentration is from about 1 to about 20%.

13. The method of claim 12 wherein the hydrostatic cyclic pressure is from about 0.05 MPa to about 3 MPa at 0.1 to about 0.5 Hz or constant pressure is from about zero to about 3 MPa above atmospheric pressure and wherein such pressure is applied  
5 for about 7 to about 28 days.

14. The method of claim 13 wherein said hydrostatic pressure is preceded or followed by a period of about zero to about 28 days of atmospheric pressure.

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15. The method of claim 14 wherein said perfusion flow rate is from about 5  $\mu\text{L}$  to about 50  $\mu\text{L}/\text{minute}$ .

16. The method of claim 15 wherein said perfusion flow  
15 rate is about 5  $\mu\text{L}/\text{minute}$ .

17. The method of claim 16 wherein said perfusion and pressure are applied at from about 2% to about 5% of oxygen concentration.

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18. The method of claim 17 wherein said neo-cartilage construct is implanted into said lesion between said two layers of the sealants.

25 19. The method of claim 18 wherein said neo-cartilage is overgrown by said superficial cartilage layer.

20. The method of claim 19 wherein said neo-cartilage and a surrounding native cartilage are mutually integrated.

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21. The method of claim 20 wherein said neo-cartilage construct comprises a thermo-reversible gelation hydrogel and is implanted into the lesion as a liquid sol wherein upon warming the construct to a body temperature, the liquid sol is  
5 converted to a solid gel and wherein this process can be reversed by cooling said lesion to a temperature below 30°C permitting removal of said gel as the sol.